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SALMON CALCITONIN STIMULATES CARTILAGE FORMATION BY INDUCTION OF COLLAGEN TYPE II AND PROTEOGLYCAN SYNTHESIS IN ARTICULAR CARTILAGE

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Purpose: Much attention has been devoted to understanding the catabolic degradation of cartilage involving enzyme activity. Much less is currently understood with regards to cartilage formation or even regeneration. Calcitonin has been shown to have beneficial effect on cartilage degradation in *in vitro* and *in vivo* pre-clinical studies. Additionally, reports show that calcitonin has anabolic effects on isolated chondrocytes. We investigated the anabolic potential of salmon calcitonin and insulin-like growth factor (IGF) in combination in an *in vitro* model of cartilage formation. We used an articular cartilage explants model, that has the extracellular matrix intact and preserves the chondrocyte phenotype compared isolated chondrocytes, and thereby may represent a more *in vivo* like system.
Methods: Articular cartilage explants was harvested from bovine stifle joints and cultured with doses of salmon calcitonin [0, 1, 10 nM] in DMEM:F12. To investigate the effects of calcitonin in combination with IGF other explants were co-cultured with salmon calcitonin [0, 1, 10 nM] plus [0.1 ng/mL] IGF. The medium was refreshed 3 times a week and the release of protein fragments were evaluated in the conditioned medium. As negative control other cartilage explants were metabolic inactivated by freeze and thaw cycles and 100 ng/mL IGF was used as positive control. Alamarblue was used to investigate the metabolic activity after the 3 weeks of culture. Collagen type II formation was quantified by PIINP ELISA measuring pro-peptides of collagen type II. Total core-protein-aggrecan turnover was measured by a G1-G2 ELISA. Sulphated and glycosylated aggrecan turnover and retention in the cartilage was measured in the alcian blue binding assay. To assess the retained proteins after 3-weeks of culture, the explants were pulverized in N_2 and the proteins solubilised with protease inhibitors. The total amount of proteoglycans was measured in the cartilage explants extracts.
Results: After 3 weeks of culture, the explants cultured as control had a lower amount of retained proteoglycans compared to the metabolic inactive explants without chondrocyte mediated metabolism, 53%, $P<0.001$. The positive control 100 ng/mL IGF increased the proteoglycan levels by 175%, $P<0.05$ compared to control. Treatment with salmon calcitonin significantly increased the content of proteoglycans in extracted cartilage explants, 1 nM by 100%, $P<0.05$ and 10 nM by 60%, $P<0.001$, compared to control. This was reflected by the quantification of released G1-G2. On the last day of culture, explants treated with 10 nM and 1 nM salmon calcitonin had 100% and 40% higher G1-G2 level compared to control, albeit only borderline statically significant. With regard to collagen type II, salmon calcitonin stimulated the collagen type II formation significantly in both doses compared to control, 1 nM by 167% and 10 nM by 129%, $P<0.05$. IGF 100 ng/mL, increased formation of collagen type II by 125%, $P<0.05$ compared to control. We further investigated possible synergistic effects of calcitonin and IGF. Simulation of articular cartilage with IGF and calcitonin, resulted in 200% increased collagen type II synthesis compared to either alone at 1 nM but not at 10 nM calcitonin, suggesting biphasic effects.
Conclusions: We show that stimulation with salmon calcitonin significantly increased the levels of extracellular matrix proteins in the cartilage. When chondrocytes were exposed to both IGF and calcitonin the anabolic response was considerably enlarged. This suggests an overlap between the anabolic pathways of IGF and calcitonin. These data begin to elucidate possible anabolic effects of calcitonin on articular cartilage.

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ACTIVATION OF MAP KINASES IN NORMAL AND OSTEOARTHRITIC HUMAN ARTICULAR CHONDROCYTES IN REPONSE TO MECHANICAL STIMULATION

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Purpose: Articular cartilage matrix synthesis and integrity are critically dependent on mechanical stimulation (MS). Human articular chondrocytes (HAC) from normal, but not from osteoarthritic (OA) cartilage, respond to cyclical MS with an increase in aggrecan mRNA and a decrease in MMP-3 mRNA1 [1]. The mitogen activated protein kinases (MAPKs) are a family of intracellular proteins that are involved in a broad range of cellular responses that lead to activation of transcription factors and gene expression in response to growth factors, cytokines and a wide variety of environmental stresses. The aim of this study was to examine the effect of MS on the phosphorylation of MAPKs in normal and OA HAC under conditions known to alter aggrecan and MMP-3 mRNA1.
Methods: Articular cartilage was obtained from patients undergoing knee joint replacement. Cartilage was graded macroscopically for OA [2]. Afer enzymatic isolation Chondrocytes were isolated and cultured in non-confluent monolayer at a density of 5×10^4/ml in Iscove's Medium with 10% FCS and 5% CO_2. The cells were mechanically stimulated for 1, 5, 10 and 20 minutes at 0.33 Hz and 3700 μstrain in the absence or presence of the p38 inhibitor SB203580 and the JNK inhibitor SP600125. Proteins were extracted and analysed by Western blotting. The membranes were probed with polyclonal rabbit phospho-specific antibodies to p38 MAPK, extracellular-signal regulated kinases (ERK1/2) and c-Jun N-terminal kinases (JNK), stripped and then reprobed for total protein with phosphorylation-status independent antibodies.
Results: p38 and ERK1/2 were identified in unstimulated human articular chondrocytes in primary culture but JNK isoforms could not be detected. Phosphorylation of p38 was increased in OA chondrocytes following 5, 10 and 20 minutes MS but there was no evidence of phosphorylation of p38 in normal chondrocytes in response to MS. Increased phosphorylation of ERK1/2 was detected in normal chondrocytes following 20 minutes MS but ERK1/2 phosphorylation was increased in only 1/3 OA primary cultures following 10 and 20 minutes MS. There was no evidence of a phosphorylated 54 kDa JNK isoform after 1, 5, 10 or 20 minutes MS in OA chondrocytes, but the polyclonal phospho-specific JNK antibody identified 44kDa and 42kDa proteins which are likely to be ERK1/2. Phosphorylation of p38 induced by MS was blocked by SB203580 at concentrations of 1μM to 10μM but paradoxically increased at higher concentrations of the inhibitor (20μM, 50μM). p38 phosphorylation was further increased in the presence of 10μM SP600125. Phosphorylated ERK1/2 and the the 44kDa and 42kDa protein bands detected with phospho-JNK antibody following MS were blocked with the SP600125 JNK inhibitor at concentrations $>10\mu$M.
Conclusions: Unstimulated normal and OA chondrocytes express p38 and ERK1/2 MAPKs in primary culture. The absence of p38 phosphorylation in normal chondrocytes following MS that leads to changes in aggrecan and MMP3 mRNA suggests that p38 is not involved in the intra cellular signaling cascade that leads to this anabolic response. The phosphorylation of p38 in OA chondrocytes following MS could be associated with altered intracellular signal transduction that results in the failure of OA chondrocytes to increase aggrecan, or decrease MMP-3 mRNA following MS [1]. The phosphorylation of ERK1/2 in normal chondrocytes following MS that leads to up-regulation of

aggrecan synthesis indicates that activated ERK does not always serve as a negative regulator of proteoglycan synthesis [3].

References

[1] Millward-Sadler J. et al, *Arthritis Rheum.* 2000; 43: 2091-2099.
[2] Collins DH and McElligott TF, *Ann. Rheum. Dis.* 1960; 19: 318-330.
[3] Starkman BG et al, *Biochem J.* 2005; 389: 723-729

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MECHANICAL COMPRESSION EFFECTS ON NO PRODUCTION AND MATRIX ALTERATION IN HUMAN OA CARTILAGE EXPLANTS UNDER DIFFERENT OXYGEN TENSIONS

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Purpose: Several factors are known to be involved in the destruction of the articular cartilage. Interleukin-1β (IL-1β) plays a determinant role in the pathogenesis of osteoarthritis (OA) by stimulating inducible NO synthase (iNOS), cyclo-oxygenase II (COX-II) and proteases. During normal activity, articular cartilage is subject to dynamic loading applied perpendicular to the cartilage surface. Compression causes deformation of cells and of extracellular matrix (ECM), gradients in hydrostatic pressure and intratissue fluid flow. These mechanical changes can also alter chondrocyte behaviour and ECM homeostasis. The present study was designed to evaluate in-vitro effects of intermittent compression on NO and GAG release by human OA cartilage explants.
Methods: Cartilage explants were exposed to intermittent compression (1 MPa, 1 Hz, 30' ON, 30' OFF) for 7 hours under normoxia (21% O_2) or hypoxia (5% O_2). NO production and GAG release in culture medium were measured for each condition. Moreover, matrix structure was analysed by second harmonic generation (SHG) imaging in a confocal microscope after multiphoton exitation.
Results: Results show that mechanical stimulation increase NO and GAG release in culture media under normoxia or hypoxia conditions. The increase of NO and GAG release in response to mechanical stimulation was more important under normoxia than under hypoxia conditions. Moreover, the collagen network was altered after compression as showed by SHG images.
Conclusions: The results suggest that oxygen tension influenced the response of cells to mechanical stimulation. The in vitro response of osteoarthritic cartilage is more important under normoxia than under hypoxia conditions. In vitro models may help to explain aspects of the interactions between mechanical forces and degradative pathways which lead to cartilage damage and disease progression.
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EFFECT OF HYDROSTATIC PRESSURE ON ULTRASTRUCTURE OF SYNOVIAL FIBROBLASTS FROM RAT TEMPOROMANDIBULAR JOINT

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Purpose: Mechanical loading of the cartilage and bilaminal zone is the important role in the pathogenesis and progression of temporomandibular disorders (TMD). However, the synoviocytes in bilaminal zone, however, the earliest pathological TMD cartilage changes are likely to result from continuous loading of joint cartilage as the patterns of TMD. This project examines the effect of hydrostatic pressure on ultrastructure of synovial fibroblastic cells from the condyle of rat temporomandibular joint.
Methods: Synovial fibroblastic cells derived from the double condyle of rat temporomandibular joint were grown to confluency in DMEM medium supplemented with 10% fetal calf serum. The monolayer of fibroblasts was then subjected to different hydrostatic pressure (30kPa, 60kPa, and 90kPa) in a computer-controlled pressure chamber or 12 h. Changes of ultrastructure were observed by transmission electron microscope.
Results: The inner-structure of normal SF was normal and intact. At 30 kPa, the ultrastructure of SF mostly shows that the chromatin was condensated lightly and ruptured to the nuclear margin. Intracellular vacuoles were observed increased visibly. At 60 kPa, the karyon takes on crescent and the mitochondria seem varicose. At 90 kPa, the apoptosis-like body was wrapped by membrane and embedded in the high density chromatin.
Conclusions: These data suggested that biomechanical stress could induce the apoptosis and result in the change of ultrastructure of synovial fibroblasts from rat temporomandibular joint.

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COMPLEX THERMAL PROPERTIES OF HUMAN CARTILAGE IN GRADE 4 OSTEOARTHRITIS

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Purpose: The purpose of this study was to further characterize the altered metabolism in human degenerated cartilage that promotes disease progression. A new protocol had to be established before the investigation. The specific causes of osteoarthritis are unknown, but are believed to be a result of both mechanical and molecular events in the affected joint. Much of what is known about changes in the extracellular matrix in osteoarthritis comes from animal models.
The change of energy in thermal processes can be measured by Differential scanning calorimetry (DSC). A limited number of papers have been published before on the subject of thermal analysis of normal and osteoarthritic human hyaline cartilage. Previously, thermogravimetric methods have not been used for compositional thermoanalytical study of normal and degenerative human hyaline cartilage.
Methods: The thermal properties of samples were determined by differential scanning calorimetry. From the DSC curves the decomposition temperature, the transition temperature range and the total calorimetric enthalpy change were calculated. The thermogravimetric analysis was performed and the TG, DTG and DTA curves were determined.
During arthroplasty procedures performed at the University of Szeged, degenerative Grade 4 human hyaline cartilage was obtained from 15 hip. All tissues were yielded in accordance to legal regulation, international ethical concerns, and patients' consent.
Results: It was found, that the total water content of the osteoarthritic samples was 86.5%, and 50kJ/M energy was used for the removal of the fluid content. In the osteoarthritic samples (average mass: 17.02 mg), 0.242 mg decrease was measured which represents 1.4% °C-1 mass reduction. The resulting amount of weight lost in the linear region was recounted from these results.
With the rise of temperature an endothermic reaction was ob-